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Microcytic Anemia With Iron Malabsorption: An Inherited Disorder of Iron Metabolism

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Two siblings were identified with severe hypoproliferative microcytic anemia and iron malabsorption, in the absence of any gastrointestinal disorder or blood loss. These children had severe microcytosis (MCV 48 fl, hemoglobin 7.5 g/dl) with decreased serum iron, elevated serum TIBC, and decreased serum ferritin, despite prolonged treatment with oral iron. An iron challenge study with an oral dose of 2 mg/kg elemental iron as ferrous sulfate documented iron malabsorption. After treatment with intravenous iron dextran, there was an absence of the expected reticulocytosis and only a partial correction of the hemoglobin, hematocrit, and microcytosis. The bone marrow was hypocellular with abnormal iron incorporation into erythroid precursor cells. This appears to be a rare form of inherited anemia characterized by iron malabsorption and disordered iron metabolism that only partially corrects after the administration of parenteral iron. These features resemble those found in the microcytic mouse (*mk/mk*), which also has severe microcytic anemia and iron malabsorption that partially responds to parenteral iron.

Key words: erythropoiesis, red blood cell disorders, iron metabolism, iron malabsorption

INTRODUCTION

The failure of oral iron therapy to correct iron deficiency anemia is often attributed to poor patient compliance with oral iron dosing regimens. Other causes may include ongoing blood losses or iron malabsorption associated with gastrointestinal disorders such as inflammatory bowel disease [1], intestinal lymphoma [2], or pancreatic enzyme supplementation [3]. Inherited disorders of iron metabolism are extremely rare in humans. In 1964, Shahidi et al. described hypochromic anemia in siblings with high serum iron concentration and hepatic iron overload [4,5]. In 1972, Goya et al. [6] described a family with congenital atransferrinemia. These patients had low serum iron, low TIBC, and severe microcytic hypochromic anemia with iron deposition in the liver, pancreas, heart, and kidneys. Buchanan and Sheenan [7] in 1981 described three siblings with iron deficiency anemia who failed to respond to oral administration of iron but did demonstrate a partial response to parenteral iron therapy. Two siblings are described here with microcytic anemia and a disorder of iron metabolism, similar to that reported by Buchanan, but with more severe anemia. These children appear to have a defect in the intestinal absorption of iron and a defect in the mobilization of iron from intracellular stores. They have maintained a low serum iron and high serum iron binding capacity after the replenishment of adequate total body iron stores. Several inbred animal strains with defects in iron absorption and metabolism have been described, and this defect appears most closely to resemble that observed in the *mk/mk* mouse [8].

CASE PRESENTATIONS AND RESULTS

History

Two African-American children (Patient AE: male age 7, and Patient JE: female age 4) were referred to the

Abbreviations: BFU-E, burst-forming units-erythroid; CFU-E, colony-forming units-erythroid; CFU-GM, colony-forming units-granulocyte monocyte; ConA, concanavalin A; FEP, free erythrocyte (zinc) protoporphyrin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red cell distribution width; TIBC, total iron binding capacity; WBC, white blood cell.

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TABLE I. Laboratory Evaluation at Presentation

	Patient JE	Patient AE	Mother	Father	Normal range[a]
WBC count	4.9	4.8	2.5	3.8	4.8–10.8 th/mm^3
% Neutrophils	29	39	39	39	37–75%
% Lymphocytes	60	47	46	46	24–44%
Platelets	584	466	198	256	130–400 th/mm^3
RBC count	5.02	5.06	4.4	5.25	4.2–5.4 mil/mm^3
Hemoglobin	7.5	7.5	12.4	14.1	12–16 g/dl
Hematocrit	24.4	24.5	37.6	41.1	37–47%
MCV	48.9	48.8	84.7	78.4	82.0–101.0 fl
MCH	14.9	14.9	27.9	26.8	27–34 pg
MCHC	30.8	30.7	33.0	34.2	32.0–36.0 g/dl
RDW	30.8	30.1	12.4	12.8	11.5–13.5%
Reticulocyte count	1.1	0.6	0.9	0.7	0.5–1.5%
Haptoglobin	96	67	—	—	14–163 mg/dl
Hemoglobin electrophoresis	AA	AA	AA	AA	Hemoglobin AA
Hemoglobin A_2	1.7	1.5	2.1	2.2	<3.5%
Hemoglobin F	<2	0.4	0.7	1.5	<2%
Ferritin	25	8.5	99	120	11.5–282 mg/L
Serum iron	17	14	57	93	48–182 mg/dl
Serum TIBC	416	397	402	433	269–450 mg/dl
% Transferrin saturation	4	3.5	14	21	>16%
Whole blood lead	6.0	6.0	—	—	0–15 mg/dl
Free erythrocyte (zinc) protoporyphrin	161	64	24	14	0–30 mg/dl
Erythropoietin (Lab 1)[b]	19	13	7	9	0–19 mU/ml
Erythropoietin (Lab 2)[b]	42.7	25	—	—	9.1–30.8 mU/ml

[a]th, thousands; mil, millions; pg, picograms.
[b]Erythropoietin levels were obtained in two separate laboratories.

Walter Reed Army Medical Center Pediatric Hematology Clinic for a lifelong history of microcytic anemia. They are full siblings, and both children have been relatively healthy. Their growth and development were normal, and they were both performing at the appropriate grade level in school for their ages. They ate a normal diet without excessive whole milk consumption. There was no history of external blood loss such as melena, hematuria, hematochezia, or hematemesis. There was no history of failure to thrive, chronic diarrhea, or other symptoms to suggest a generalized malabsorptive state. There was no history of lead exposure or exposure to other bone marrow toxins. Patient AE was diagnosed with anemia during an evaluation prior to surgery for craniosynostosis. Because of his diagnosis his sister was followed after her birth for the possible development of microcytic anemia. Both patients had been treated with several courses of iron as ferrous sulfate (Patient AE: 6 courses, Patient JE: 4 courses) at a dose of 6 mg/kg/day of elemental iron for 30–60 days for each course. The patients' mother asserted that she had given all of the prescribed courses of oral iron treatment.

Family History

There was no history of consanguinity. The maternal grandmother had chronic anemia, and was treated with oral iron for many years, until hysterectomy about 10 years ago. A maternal aunt had required intramuscular iron shots during both of her pregnancies. She had a hysterectomy about 3 years ago, but remained iron deficient, with a poor response to an oral iron absorption test (see below).

Physical Examinations

Both patients had normal physical examinations. Patient AE had a surgical scar on his scalp, from his previous surgery for craniosynostosis. They had no organomegaly or skeletal defects. There were no changes in their fingernails or oral mucosa (e.g., koilonychia, glossitis, angular stomatitis, associated with severe iron deficiency) [9]. Growth curves were normal for both children.

Laboratory Studies

Laboratory studies are presented in Table I. The peripheral blood smears for both children were abnormal, as shown in Figure 1. Stools were negative for occult blood on three occasions for each child. An abdominal ultrasound revealed normal spleen size and no evidence of gallstones, for both children. Hemoglobin electrophoreses were normal for both patients and for their parents (Table I). In addition, no hemoglobin H inclusions were seen with a brilliant cresyl blue stain of fresh erythrocytes. Serum ceruloplasmin levels were normal. The patients' red blood cells had increased resistance to osmotic hemolysis, in contrast to the increased osmotic fragility found in hereditary spherocytosis. There was normal upregula-

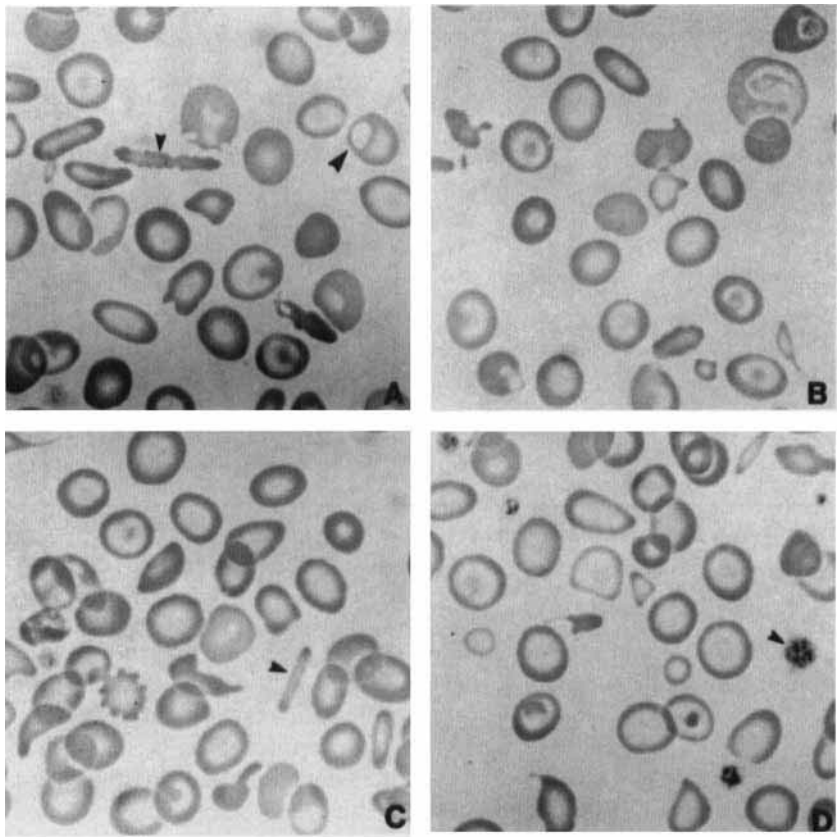

Fig. 1. Peripheral blood. Blood smear from Patient AE, prior to iron therapy, shows anisocytosis, poikilocytosis, microcytosis (MCV = 48), hypochromasia, and schistocytosis. A, C: The erythrocyte "pencil cell" shape may be found in severe iron deficiency (arrows). A–D: Target cells, elliptocytes. A: Blister cells (large arrow). D: Large platelets (arrow). The peripheral blood for Patient JE appeared similar, and morphologic abnormalities partially improved after parenteral iron therapy for both children.

tion of transferrin receptors on blood lymphocytes after 3 days in culture with ConA stimulation [10].

Oral Iron Challenge Study

Both children were challenged with an oral dose of iron as ferrous sulfate at a dose of 2 mg/kg [11], and serum iron values from the next several hours are shown in Figure 2. Although both children show evidence of some iron absorption, the rise in serum iron is well below that expected for children with iron deficiency. The average rise in serum iron, in a study of iron-deficient children given 1 mg/kg elemental iron as ferrous sulfate was 274 μg/dl [11]. The blunted rise in serum iron after an oral dose of 2 mg/kg elemental iron as ferrous sulfate is consistent with iron malabsorption.

In addition, the maternal aunt underwent an oral iron challenge study as part of an evaluation for iron deficiency. Before the study, her hematologic laboratory values included hemoglobin 10.4 g/dl, hematocrit 31.8%, MCV 68 fl, MCH 22.8 pg, MCHC 32.6 g/dl, serum iron 10 mg/dl, TIBC 400 mg/dl, and ferritin 14 mg/L. After

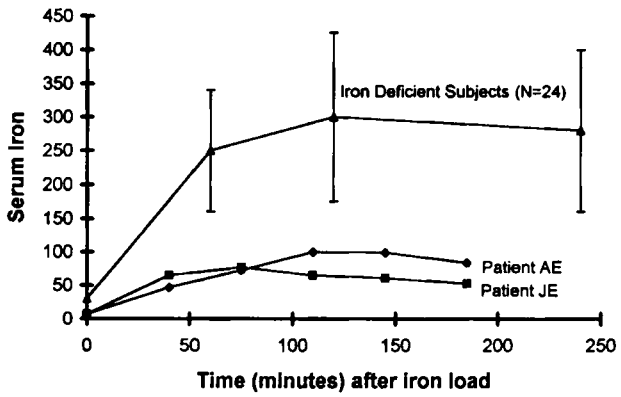

Fig. 2. Oral iron challenge study. The amount of oral iron given at time 0 was 2 mg/kg of elemental iron as ferrous sulfate per child. The expected normal response range, derived from 24 iron-deficient children given an oral dose of 1 mg/kg of elemental iron, is also shown [11].

TABLE II. Laboratory Values After Parenteral Iron Dextran*

	Interval									
	0[a]	2 wk	1 mo	2 mo	4 mo	6 mo[b]	8 mo	10 mo[c]	18 mo[d]	21 mo
Hemoglobin (g/dl): JE	7.5	8.2	8.9	8.3	8.9	8.7	9.5	9.1	8.6	9.9
Hemoglobin (g/dl): AE	7.5	8.6	8.1	8.3	9.0	9.7	8.9	8.9	9.1	9.6
Hematocrit (%): JE	24.4	25.7	28.8	27.1	28.1	28.0	29.6	29.7	26.7	30.7
Hematocrit (%): AE	24.5	26.7	26.2	27.1	28.0	30.6	27.9	28.8	29.1	29.4
MCV (fl): JE	48.9	51.8	54.0	55.6	54.5	54	56.5	57.1	55.5	60.1
MCV (fl): AE	48.8	51.6	53.0	54.3	54.4	53.2	55.2	56.2	54.5	58.1
Reticulocytes (%): JE	1.3	0.4	2.6	0.7	—	—	—	—	0.6	1.2
Reticulocytes (%): AE	0.5	0.9	1.4	0.4	—	—	—	—	0.3	0.8
Serum iron (mg/dl): JE	7	6	—	13	—	7	—	—	21	<25
Serum iron (mg/dl): AE	7	14	—	20	—	7	—	—	14	32
TIBC (mg/dl): JE	380	301	—	375	—	336	—	—	—	296
TIBC (mg/dl): AE	433	368	—	415	—	405	—	—	314	322
Ferritin (mg/L): JE	28.3	—	—	148	—	134	187	211	153	297
Ferritin (mg/L): AE	8.7	—	—	62	—	87	68	58	67	144

*Intravenous iron dextran doses, as mg of elemental iron, were given to each child at the intervals noted: [a]400 mg; [b]200 mg; [c]200 mg (Patient AE only); [d]400 mg.

an oral dose of 200 mg of elemental iron as ferrous sulfate (fer-in-sol), the serum iron rose from 10 mg/dl to only 76 mg/dl at 3 hr.

Parenteral Iron Dextran

Both children were given parenteral iron dextran 400 mg by intravenous (IV) infusion over 4 hr. This amount of iron was calculated to replenish total body iron stores for both patients. Laboratory values taken before and after these infusions are shown in Table II. After intravenous iron, there was a partial correction of the hemoglobin, hematocrit, and MCV; however, the response was distinctly different than would be expected for iron deficiency alone. There was not the expected reticulocyte response, and the serum iron and TIBC remained decreased although the serum ferritin increased. Subsequently both children have been given additional infusions of intravenous iron dextran, as shown in Table II. There appears to be a trend toward higher hemoglobin and MCV values following additional iron dextran infusions, and associated with elevated ferritin levels.

Bone Marrow Examination

Bone marrow examinations were performed on both patients 4 months after the first dose of intravenous iron dextran, as shown in Figure 3. Bone marrows for both patients had about 50% cellularity, which was unexpectedly hypocellular for age and for the degree of anemia. There was a relative erythroid hyperplasia with adequate trilineal maturation. The erythroid elements had irregular cytoplasmic borders and some intercytoplasmic strands, as shown in Figure 3 (A–C). A small amount of iron (1+ out of 6+) was seen in marrow macrophages from Patient JE, and a trace amount seen (0-1+ out of 6+) in marrow macrophages from Patient AE. A slight difference in body weight in relation to quantity of parenteral iron previously infused may account for the slight difference in storage iron seen between the two patients. In contrast to the marrow macrophages, the erythroid precursor cells did not contain normal siderotic granules. Rare erythroblasts contained an amorphous collection of Prussian-blue staining material in the cytoplasm that did not match the pattern of siderotic granules or siderotic rings (not shown).

Bone Marrow Culture Studies

Bone marrow culture studies were performed at the time of the bone marrow aspirates. Burst-forming units-erythroid (BFU-E) and colony-forming units-erythroid (CFU-E) were determined following standard methods [12] in the presence of increasing concentrations of erythropoietin (Fig. 4). Erythroid colonies appeared normal in size, morphology, and hemoglobinization. Numbers of erythroid colonies formed at each erythropoietin concentration were increased for both patients, compared to erythroid colonies formed from bone marrow from four normal healthy adults. Also of interest, the patients had lower numbers of CFU-GM colonies, representing committed myeloid precursor cells, at each erythropoietin concentration, than did the controls (data not shown). Bone marrow from normal adult donors was collected according to a protocol approved by the Walter Reed Army Medical Center Human Use Committee.

Erythropoietin

Serum erythropoietin levels were relatively low for the degree of anemia (Table I). Because of the good response of erythroid progenitors in bone marrow culture, erythropoietin was given to each child at a dose of 50 U/kg SC three times weekly for a period of 3 weeks, followed by

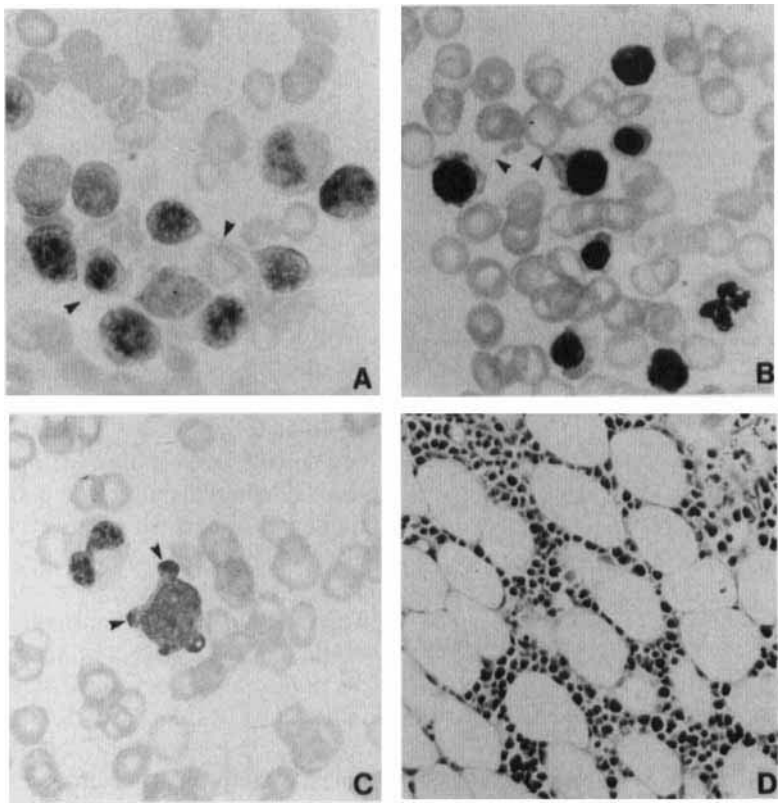

Fig. 3. Bone marrow, Patient AE. Erythroid precursors are poorly hemoglobinized. A, B: Some late normoblasts have cytoplasmic stranding between cells (arrows). C: Early erythroid precursor cells have cytoplasmic blebs (arrows). These nonspecific features are not found in iron deficiency. D: Biopsy revealed relative marrow hypoplasia for age and for degree of anemia. The bone marrow was obtained 4 months after the first parenteral iron therapy. Bone marrow aspirate and biopsy from Patient JE appeared similar.

an increase to 100 U/kg three times weekly for an additional 3 weeks. Hemoglobin values rose slightly for each child: 9.1–9.7 g/dl for Patient JE and 8.9–9.8 g/dl for Patient AE; MCV values remained unchanged at 57 fl. Erythropoietin therapy was then discontinued due to the minimal response.

DISCUSSION

These patients have a hypoproliferative anemia manifested by a low reticulocyte count and hypocellular bone marrow that does not appear to be a thalassemic condition. Their hemoglobin electrophoreses were normal, and they did not have splenic enlargement, which is frequent in conventional forms of thalassemia. Furthermore, their bone marrows had decreased cellularity for children of this age, especially in the presence of anemia. Thalassemia normally has a hypercellular bone marrow, as does iron deficiency [13]. In addition to the red blood cell abnormalities, both the patients and their parents have borderline leukopenia and neutropenia (Table I). The significance of this finding is unclear, since this degree of leukopenia may be normal in African Americans.

The presence of severe iron deficiency anemia in patients eating a normal diet, without hidden blood loss and despite repeated courses of oral iron supplements, is distinctly unusual and suggested a specific defect of intestinal iron absorption. This was documented by an oral iron challenge test (Fig. 2). But even after parenteral iron was given as intravenous iron dextran, the serum iron and transferrin saturation failed to normalize. Moreover, the bone marrow iron stain, after iron therapy, failed to show normal sideroblasts (erythroid normoblasts with visible aggregates of iron in the cytoplasm), despite the presence of stainable iron in the marrow macrophages. Taken together, it seems that these patients have a defect in iron metabolism, including intestinal iron malabsorption, abnormal mobilization of storage iron, and possibly a defect in the uptake of iron by erythroid marrow elements. The key features of this condition are microcytic anemia and iron deficiency that does not respond to oral iron supplements. Furthermore, parenteral iron therapy results in a delayed response and only partial correction of the anemia and microcytosis.

These patients are similar to three siblings described by Buchanan and Sheenan [7] in 1981; however, the

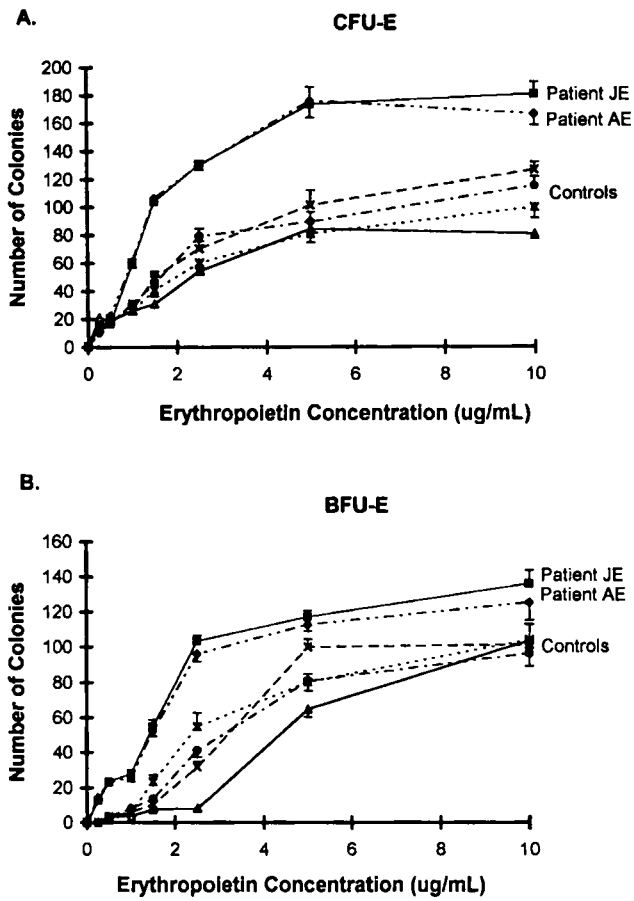

Fig. 4. Growth of erythroid progenitor cells in bone marrow culture. CFU-E are colonies formed from relatively mature erythroid cells, while BFU-E are colonies derived from primitive, immature erythroid progenitors. The growth of both CFU-E (A) and BFU-E (B) were increased in bone marrow culture from the two patients in response to varying concentrations of added erythropoietin. The results are compared to cultures of bone marrow obtained from four normal individuals. Results represent the mean ±SEM for quadruplicate cultures for each condition.

anemia observed in our patients is more severe. Our patients have morphologic abnormalities of the red blood cells that were not described in Buchanan's patients. Also, our patients have a lower MCV, both initially and after parenteral iron, compared to the patients described by Buchanan. In Buchanan's patients, the MCV values started at about 57 fl and rose to 65 fl, while for our patients, the MCV values started at 48 fl and rose to 58–60 fl after parenteral iron treatment. Buchanan's patients appear to be the only other similar cases in the medical literature reported to date, and may represent a milder clinical pattern of the same condition.

In vitro bone marrow culture studies (Fig. 4) were performed because we believed that these patients may have had a defect in erythroid colony formation. Unexpectedly, the patients had increased BFU-E and CFU-E at all levels of erythropoietin supplementation. Furthermore, the patients had low serum erythropoietin levels for their degree of anemia. These findings led us to attempt a trial of erythropoietin in our patients, but they did not have a clinically significant response. This could have been due to the persistent decrease in serum iron despite adequate storage iron, or to an underlying bone marrow iron uptake defect that could not be overcome in vivo.

The red blood cells (RBCs) of the patients' father have a borderline microcytosis (Table I), unlike the RBCs of the parents of the children reported by Buchanan and Sheenan. In addition, the maternal grandmother and maternal aunt have had documented iron deficiency anemia as adults despite multiple courses of oral iron therapy, and the maternal aunt had a poor response to an oral iron challenge test in the presence of serologic evidence of iron deficiency. These findings suggest that a mild iron absorption defect is present in some maternal relatives. The mode of inheritance may include partial penetrance of the metabolic defect in this family, but the severity of the children's defect suggests that the primary mode of inheritance is autosomal recessive.

Inherited disorders of iron metabolism are rare in humans, although several inbred animal strains have been found to have defects in the intestinal uptake, transport, or storage of iron [8]. There appears to be a close analogy between the condition described here and a recessive trait in mice, named microcytic anemia (gene symbol *mk*) [14]. The features of iron deficiency anemia are present but the anemia fails to respond to oral iron therapy, and the response to iron dextran injection is at best partial. Iron absorption by intestine [15] and uptake by reticulocytes [16] is impaired, leading to the hypothesis of a general impairment of iron entry into cells [17]. Microcytosis persists when *mk/mk* stem cells are transplanted into normal irradiated hosts [18], demonstrating that the lesion is inherent to stem cells, as well as other tissues. We did not directly measure iron uptake by erythroid bone marrow elements from our patients, so we cannot confirm that the defect is similar at this level. Additional studies of the defects in *mk/mk* mice, and in the patients described here, will be needed in order to elucidate the molecular basis of these disorders.

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